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CONTRASTING GENETIC STRUCTURE OF ADULTS AND PROGENY IN A LOUISIANA IRIS HYBRID POPULATION

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Abstract.—Studies of natural hybridization have suggested that it may be a creative stimulus for adaptive evolution and speciation. An important step in this process is the establishment of fit recombinant genotypes that are buffered from subsequent recombination with unlike genotypes. We used molecular markers and a two-generation sampling strategy to infer the extent of recombination in a Louisiana iris hybrid zone consisting predominantly of *Iris fulva*–type floral phenotypes. Genotypic diversity was fairly high, indicating that sexual reproduction is frequent relative to clonal reproduction. However, we observed strong spatial genetic structure even after controlling for clonality, which implies a low level of pollen and seed dispersal. We therefore used cluster analysis to explore the hypothesis that the *fulva*-type hybrids are an admixture of groups between which there has been limited recombination. Our results indicate that several such groups are present in the population and are strongly localized spatially. This spatial pattern is not attributable strictly to a lack of mating opportunities between dissimilar genotypes for two reasons: (1) relatedness of flowering pairs was uncorrelated with the degree of overlap in flowering, and (2) paternity analysis shows that pollen movement among the outcross fraction occurred over large distances, with roughly half of all paternity attributed to pollen flow from outside the population. We also found evidence of strong inbreeding depression, indicated by contrasting estimates of the rate of self-fertilization and the average inbreeding coefficient of *fulva*-type hybrids. We conclude that groups of similar hybrid genotypes can be buffered from recombination at small spatial scales relative to pollen flow, and selection against certain recombinant genotypes may be as important as or more important than clonal reproduction and inbreeding.

Key words.—Inbreeding depression, mating patterns, natural hybridization, paternity analysis, reproductive isolation, spatial autocorrelation.

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Numerous investigators have examined the dynamics of natural hybridization to identify the nature of species boundaries. These studies show that species boundaries are eclectic phenomena that may have simple or complex genetic and ecological foundations (reviewed by Templeton 1981; Orr 2001; Howard et al. 2002). Furthermore, species boundaries may be semipermeable, allowing the introgression of genetic material from one species to another (Key 1968; Harrison 1990). An extensive literature has developed to address the theoretical basis and empirical pattern of introgression in a diverse array of organisms (Endler 1977; Barton and Hewitt 1985; Harrison 1990; Arnold 1997). These studies seek to draw general conclusions regarding the evolutionary consequences of hybridization from detailed study of dispersal, selection, and mating patterns.

Such work has led to the hypothesis that selection may favor the introgression of certain traits, and can result in the transfer of complex adaptations and even the creation of ecologically distinct homoploid (i.e., nonpolyploid) species (Anderson and Stebbins 1954; Lewontin and Birch 1966). Indeed, recent studies have documented the occurrence of natural hybrids in habitats outside the normal range of parental types (e.g., Cruzan and Arnold 1993) as well as greater fitness of hybrid genotypes in novel or extreme environments (e.g., Lexer et al. 2003). Molecular techniques have been used to verify species of hybrid origin (e.g., Rieseberg 1991; Arnold 1993; Wolfe et al. 1998), and the genetic architecture of adaptation via hybridization has been the topic of recent investigations (Rieseberg et al. 2003).

There are, however, theoretical difficulties with the establishment of novel recombinant lineages. The primary obstacle is the breakup of rare advantageous gene combinations by subsequent recombination with the more abundant parental types. Several authors have investigated models that address the likelihood of adaptive introgression and/or hybrid speciation (Grant 1971; Templeton 1981; McCarthy et al. 1995; Buerkle et al. 2000; Barton 2001), usually in the context of recombinational speciation (Grant 1971). These models demonstrate the feasibility of hybrid speciation under some combination of chromosomal or genic sterility, self-compatibility or asexual reproduction, and ecological isolation. More empirical studies of hybridization are needed to determine whether these conditions are frequently realized in nature, or indeed if empirically demonstrated cases conform to these predictions.

A number of complementary strategies can be employed to study the outcomes of hybridization. Mechanistic studies of prezygotic and postzygotic barriers have been used to infer the rates of initial and subsequent hybrid formation, and to identify conditions under which hybridization is most likely to occur (e.g., Grant and Grant 1996; Hodges et al. 1996; Campbell et al. 1998). Common-garden studies of known or artificially produced hybrid classes have been used to evaluate the performance of early-generation hybrids in various environments (e.g., Emms and Arnold 1997; Wang et al. 1997; Burke et al. 1998a; Orians et al. 1999; Schweitzer et al. 2002). A third approach that has grown in utility over the past few decades is the use of molecular markers to infer population-genetic processes from the distribution of genotypes in natural populations (e.g., Arnold et al 1987; Szymura

and Barton 1991). Molecular markers are useful tools because they provide more lucid descriptions of the structure of hybrid zones, which are often admixtures of individuals with complex ancestry, and because they can be used to infer patterns of gene exchange, recombination, and selection. One goal of marker-based studies is to use patterns of genetic structure at different life-history stages to predict how the genotypic constitution of hybrid zones may change over time due to these factors (e.g., Cruzan and Arnold 1994 and references therein). This cross-generational approach is made more powerful if phenotypic traits that affect mating are recorded, so that potential genetic correlations induced by mate choice can be identified and not erroneously attributed to postzygotic selection. Molecular markers can thus be used to infer population-level processes relevant to the likelihood of adaptive introgression or hybrid speciation.

Here we use the latter approach to investigate sources of genetic structure in a Louisiana iris hybrid zone. The factors shaping the distribution of genotypes in adults and progeny have been examined previously in Louisiana iris hybrids by Cruzan and Arnold (1994). The authors used species-specific nuclear and chloroplast markers for *Iris fulva* and *I. brevicaulis* and a spatially restricted mixed-mating model to generate expected distributions of seed genotypes. By comparing this expected distribution with the observed distribution of progeny, they concluded that there was assortative mating among *I. fulva* cytotypes (i.e., *I. fulva* and ''*I. fulva*–like'' hybrids) resulting in progeny with more *fulva* markers than expected. This was not observed for *I. brevicaulis* cytotypes. Furthermore, seed progeny that had intermediate genotypes were aborted at higher rates. Thus, both assortative mating and postfertilization selection were detected by this study. However, this assortative mating occurred among fairly differentiated groups, *I. fulva* cytotypes versus *I. brevicaulis* cytotypes, because intermediate forms were lacking among adult plants. Due to this, the study lacked the resolution to determine how much gene flow occurred among genets of a given cytotype. It is this latter question that is most relevant to the stabilization of novel adapted lineages, because such matings are less constrained by prezygotic or postzygotic factors.

In the present study, we used molecular markers to compare the genetic structure of adult plants and their seed progeny produced during one reproductive season in a Louisiana iris hybrid zone. We also recorded the dates of opening and closing of each flower in the population to determine whether overlapping phenology leads to genetic correlations among mating pairs. From these combined data, we then inferred the extent to which phenology, postzygotic selection, and/or dispersal direct hybridization outcomes. Specifically, we asked the following questions: (1) What is the genetic structure of the adult population in terms of inbreeding coefficients, linkage disequilibrium, and the spatial distribution of alleles? (2) Are coflowering individuals genetically correlated? (3) What is the distribution of family-level outcrossing rates? (4) What is the genotypic distribution of progeny, and what is the distance of pollen movement? (5) Are the observed selfing rates and patterns of pollen flow consistent with the observed genetic structure of the population?

Materials and Methods

Study Organism and Sampling

The Louisiana irises have long served as a model system for studying hybridization in plants (Riley 1938; Anderson 1949; Randolph 1966; Bennett and Grace 1990; Arnold 2000). This North American species complex (section Hexagonae) consists of four accepted species: *Iris fulva, I. brevicaulis, I. hexagona,* and *I. nelsonii* (we follow Foster [1937] and Goldblatt [1990] in maintaining *I. hexagona* var. *giganticaerulea* at subspecific rank). *Iris fulva, I. brevicaulis,* and *I. hexagona* have broad but largely distinct ranges in the central and eastern United States. These distributions overlap in southern Louisiana where hybridization is frequent. The fourth species, *I. nelsonii,* is itself a stabilized hybrid of the other three (Randolph 1966; Arnold 1993), and is a rare endemic of southern Louisiana. The species are distinctive in terms of pollination biology and ecophysiology, but hybrids often have high fitness under experimental conditions (Johnston et al. 2003) and in nature can be found in both parental and novel environments (Johnston et al. 2001). This system is therefore ideal for investigating the creative role of hybridization in producing new adaptations and/or new species.

The Louisiana iris species are perennial, spring-flowering herbs that also reproduce vegetatively by budding of underground rhizomes. Because of this clonal habit, we shall use the term ramet to identify physically separate individual plants and the term genet to identify the collection of clonally derived ramets that are ultimately the same genetic individual. Two very distinct pollinator syndromes are present in the Louisiana irises. The red-flowered *I. fulva* is predominantly visited by hummingbirds, whereas the blue-flowered *I. hexagona* and *I. brevicaulis* are visited primarily by bumblebees. While there is a small amount of overlap in pollinator visitation between the species, the efficacy of these visits in effecting pollination is unknown and the frequency of F_1 seed formation in nature is very low (Arnold et al. 1993, Hodges et al. 1996). Although the species are self-compatible, flowers are protandrous for one to two days after anthesis, after which the stigma surface is exposed and receptive (Cruzan and Arnold 1994).

The studied population borders Young's Coulee (a bayou) in Vermillion Parish, southern Louisiana. Although we have limited knowledge of the history of this population, Louisiana iris hybrids have persisted in the area for decades, if not longer (Randolph et al. 1961). The population consists of dense patches of ramets growing on a mudflat and adjacent shaded banks of the bayou. The site has been previously investigated (Arnold 1993) and nuclear markers for *I. fulva, I. brevicaulis,* and *I. hexagona* and chloroplast markers for *I. fulva* and *I. hexagona* were detected. Most plants have *I. fulva* cytotypes and are morphologically and genetically hybrid. There is also a single patch of ramets that is morphologically *I. hexagona,* but within which *I. fulva* nuclear markers have also been detected (Arnold 1993). We therefore consider two broad phenotypic classes of iris to be present: *fulva*-type hybrids and *I. hexagona*. Mating between phenotypic classes is expected to be rare due to prezygotic barriers such as low pollinator overlap and pollen competition (reviewed in Arnold 2000). Although quantitative variation in floral traits

exists among *fulva*-type hybrids in nature (R. Cornman, unpubl. data), we do not know to what extent, if any, this variation affects mating patterns within this phenotypic group. However, Wesselingh and Arnold (2000) have shown that hummingbirds do not discriminate against a broad range of hybrid phenotypes.

The Young's Coulee population is bordered by pasture, housing, and swamp and is separated from most other patches of ramets by 50 m or more. In the spring of 1997, all ramets were spatially mapped to the nearest centimeter. The mapped area was approximately 60 by 25 m and contained 176 *fulva*-type ramets and 21 *I. hexagona* ramets. A small number of ramets were located within 50 m of the circumscribed population but could not be mapped for logistical reasons.

The study site was visited almost daily from March 30 to April 24, and the gender phase of each flower was recorded from anthesis until wilting. In a few instances of missing observations, the status of flowers was estimated by assuming that stigmas become receptive one day after anthesis. This assumption is conservative with respect to the exclusion of candidate mating pairs. As is typical for these species, most ramets produced a single inflorescence; however, two ramets each produced two separate inflorescences. Reproductive output was relatively low (cf. Wesselingh and Arnold 2003), presumably due to drought conditions. We therefore collected three fruits from two additional *fulva*-type hybrids located near the mapped population to improve our estimate of the mating system and pollen flow. These plants were not included in any genetic structure analysis of adults.

The average number of flowers per inflorescence was 3.1 $\pm$ 0.9 (SD), the percentage of flowers setting fruit was 29% (23 of 80), and the average number of seeds per fruit was 19.0 $\pm$ 14.2 (SD). Fruits were allowed to mature naturally prior to collection and transported to the University of Georgia in June 1997. After fruits had dried in the laboratory, seeds were counted and then germinated in soil. Leaf tissue of germinated progeny was harvested for DNA extraction. Seeds that failed to germinate after six months were deemed inviable. Tissue was stored at -80°C prior to extraction and genotyping in 2001 and 2002.

DNA Extraction and Genotyping

Genomic DNA was extracted using a hexadecyltrimethyl ammonium bromide (CTAB) extraction buffer protocol (Doyle and Doyle 1987). All ramets and all progeny were genotyped at five microsatellite loci characterized by Burke and Arnold (1999). Polymerase chain reaction (PCR) was performed in a 20 μl volume containing 8 pmol each of a fluorescently labeled forward primer and an unlabeled reverse primer, 0.8 units of *Taq* polymerase in the supplied buffer (Promega Corp., Madison, WI), 2.0 mM magnesium chloride, 125 μM of each deoxyribonucleotide triphosphate, and approximately 500 ng of genomic DNA. Reactions were performed with a Perkin Elmer (Wellesley, MA) model 9700 thermocycler. The reaction products were separated on a 6% polyacrylamide gel using an ABI Prism 377 electrophoresis unit (Applied Biosystems, Foster City, CA), and allele sizes were estimated using the GeneScan software package (Applied Biosystems).

Two of the five primer pairs, IB025 and IB141, had segregation patterns in progeny arrays indicating a high frequency of null alleles, and the allelism of bands could not be reliably ascertained. Because strong linkage disequilibrium exists in this population (see Results) and because these loci had low polymorphism and effectively dominant expression, they added negligible information and were discarded from further analysis. A third primer pair, IF073, amplified multiple loci with overlapping allele sizes, as indicated by non-Mendelian segregation and the presence of individuals with up to five alleles. Our attempts to redesign the primers to isolate a single locus were not successful. We therefore treated this locus as a dominant DNA fingerprint and used it to eliminate candidate males in the analysis of paternity; however, we removed this locus from analyses of genetic structure (see below).

Prior to the availability of these microsatellite markers, the mapped ramets were genotyped at seven isozyme loci by Burke et al. (2000) to investigate clonal structure in this and another hybrid population. We have included the Young's Coulee isozyme data of Burke et al. (2000) in the present analysis of adult population genetic structure. However, we have not repeated analyses reported in that paper except where relevant to the present study.

Analysis of Genetic Structure

Our primary goal in this study was to describe spatial genetic structure and mating patterns within the *fulva*-type phenotypic class, because matings between phenotypic classes are expected to be rare. Thus, we performed the analyses described below on each of two datasets, the complete population and the subset of *fulva*-type hybrids.

Each locus was tested independently for deviations from Hardy-Weinberg equilibrium using the exact test of Guo and Thompson (1992) computed with GenePop (Raymond and Rousset 1995). Genotypic linkage disequilibrium was evaluated by calculating the common correlation coefficient of alleles with the program LinkDos (Garnier-Gere and Dillmann 1992) assuming independence of each pairwise comparison. Statistical significance was tested using the χ^2 method of Weir (1979) implemented by LinkDos. The program GenAlEx (Peakall and Smouse 2001) was used to test for spatial autocorrelation of alleles at successive two-meter distance classes, with confidence intervals generated by 999 iterations of bootstrap resampling. We also calculated the residual spatial autocorrelation not attributable to clonal structure by including a single ramet per genet positioned at the center of the genet, that is, the mean X and Y coordinates of all ramets.

Spatial autocorrelation is a statistically powerful measure of average spatial structure, but such summary measures may obscure biologically relevant detail. We therefore included additional exploratory analyses of spatial structure. Our first method was to quantify the degree to which particular genotypic classes were spatially restricted in their distribution relative to the population as a whole. For each genet, we determined the center as described above, from which we calculated the average radial distance of ramets. We then used a *t*-test to compare the average radial distance within clones

versus between clones, with degrees of freedom adjusted for unequal variances using SPSS 11.0 (SPSS Inc., Chicago, IL). The radial dispersal of each single-locus genotype and each allele present in at least three genets was calculated analogously.

The second approach was to use the model-based clustering method of Pritchard et al. (2000), which parameterizes the degree to which individuals in a sample are admixtures of individuals derived from genetically differentiated source populations. In the context of a hybrid zone, the clusters can be considered subsets of individuals within which there has been random mating, and individuals of mixed ancestry can be identified. The method is implemented by the program Structure (Pritchard et al. 2000) and does not require prior knowledge of the actual number of source populations or their gene frequencies. These are inferred by clustering samples into groups that minimize Hardy-Weinberg and linkage disequilibria, without reference to their spatial location in the population. The Structure simulation was run using the admixture model with correlated allele frequencies for 100,000 iterations after a burn-in of 10,000 iterations. The correlated allele frequencies option is preferable for investigating structure in less-differentiated populations (Pritchard et al. 2000), although there was no appreciable difference in our results if uncorrelated frequencies were assumed. For the most probable value(s) of *K*, we then mapped individuals according to their assigned group and characterized the extent to which the inferred groups are spatially clustered using spatial autocorrelation. The posterior probabilities of *K* were determined by Bayes's rule using a uniform prior probability for all values of *K*, although these probabilities should be considered rough approximations (Pritchard et al. 2000).

An assumption of the Structure model is that the presumed source populations are approximately randomly mating. This assumption is not likely to hold strictly for these species (e.g., Cruzan et al. 1994), and this departure from the model will tend to inflate estimates of *K*, the number of randomly mating groups (Pritchard et al. 2000). However, the objective of this analysis is not to determine the exact number of ''true'' clusters, but to approximate the degree to which recombination has occurred in a spatial context. A second difficulty is the presence of clonal genetic structure, which can generate linkage disequilibrium among ramets even if the underlying genets are in linkage equilibrium. We therefore performed the Structure analysis on genets only, but included all ramets in the spatial autocorrelation of group identity.

Effect of Phenology on Genetic Assortative Mating

Louisiana iris species vary in their phenology (Cruzan and Arnold 1993), which is a potentially important factor determining hybridization dynamics. Furthermore, phenology varies among artificial *I. fulva* × *I. brevicaulis* hybrids and is heritable (A. Bouck, unpubl. data). It is therefore possible that in nature similar genotypes overlap in flowering to a greater extent than dissimilar genotypes, thereby retarding the rate of recombination and potentially serving as a mechanism preserving novel lineages.

To examine the effect of phenology on the genetic correlation of mating pairs, we first determined the relatedness, as defined by Queller and Goodnight (1989), of flowering individuals relative to the population as a whole. The program Relatedness 5.0 (http://www.gsoftnet.us/Gsoft.html) was used to calculate relatedness values. Within the cohort of flowering individuals, the effect of phenology on genetic assortative mating was assessed in two ways. First, we examined the genetic distance between individuals relative to the date of first flowering. This phenological trait has moderately high heritability under greenhouse conditions (A. Bouck, unpubl. data) and may be the most relevant to mating patterns in nature, because the first flower in *I. fulva* contributes disproportionately to seed set (Wesselingh and Arnold 2003). We calculated genetic distances (Φ_{PT}, Smouse and Peakall 1999) between all flowering individuals. These values were then regressed on the pairwise difference, in number of days, between the respective dates of first flower using a Mantel test. The second method weights pairwise comparisons by the total number of mating opportunities (i.e., pairwise fertilities), which we here define as the total number of distinct pairs of male-phase and hermaphrodite-phase flowers open on a given pair of ramets on a given day. Thus, the weight for each flowering pair *ij* is calculated as (the number of flowers open on *i* times the number of flowers with receptive stigmas on *j*) + (the number of flowers open on *j* times the number of flowers with receptive stigmas on *i*), summed over all days of overlap. This weight is symmetric with respect to each member of a pair, so that a Mantel test can be used to correlate a matrix of weights and a matrix of genetic distance. It should be noted that the per diem component of the weight increases geometrically with flower number, and thus mating opportunities increase nonlinearly with greater phenological overlap. We therefore compute the Mantel regression after adding one to each weight and taking the natural logarithm. Overall, this method of correlation includes more information about potential mating pairs than does the regression of genetic distance on date of first flower. However, since flowers at different locations in the inflorescence are not equally likely to set seed (Wesselingh and Arnold 2003), the method is not necessarily a more precise way to assess whether phenology creates genetic structure in progeny arrays. Rather, the methods are complementary and we present both for completeness. The program GenAlEx (Peakall and Smouse 2001) was used to calculate genetic distances and to perform the Mantel tests.

Analysis of Mating System and Progeny Genetic Structure

The total outcrossing rate of each fruit was calculated simply as the number of apparent outcross seeds divided by the total number of seeds. The minimum number of fathers contributing to each fruit was determined manually from the total number of unique outcross alleles detected. We did not incorporate a maximum-likelihood correction for the probability of undetected outcross events (see Ritland 2002) for three reasons. First, sample sizes were small for some fruits and pollen allele frequencies were expected to differ among families, conditions that limit the robustness of maximum-likelihood estimation (Ritland 2002). Second, the rate of detectable outcrossing per family was generally very high or very low, and thus correcting for cryptic outcrossing should not change mating system estimates appreciably. Third, avail-

TABLE 1. Allelic diversity of microsatellite loci. Expected heterozygosities (H_e) were calculated using Cervus (Marshall et al. 1998). Null frequency in the total population was estimated using GenePop (Raymond and Rousset 1995). Null frequency among flowering individuals was estimated manually from segregation patterns in fruits. Only the total number of alleles amplified by the IF073 primer pair is presented, because at least two overlapping loci are present and each allele is treated as a dominant locus for use in paternity analysis (see text).

	Total population				Flowering individuals			
Locus	Alleles	H_e	H_o	Null frequency	Alleles	H_e	H_o	Null frequency
IB145	10	0.849	0.741	0.101	9	0.840	0.920	0.042
IF061	9	0.810	0.894	0.026[1]	8	0.781	0.913	0.125[1]
IF073	7	—	—	—	7	—	—	—

[1] All *Iris hexagona* genets are null at locus IF061.

able estimation methods do not allow mixture of dominant and codominant markers.

We used a fractional method of paternity assignment (Schoen and Stewart 1986; Devlin et al. 1988), which is particularly appropriate for quantifying population-level parameters such as pollen flow (Devlin et al. 1988). For each flower, candidate pollen donors were identified from their observed dates of flowering. Candidate males were then tested for compatibility with each mother-offspring pair at the two codominant microsatellite loci, using the software package Cervus (Marshall et al. 1998). The remaining candidate males were then examined manually at the dominant marker IF073. Paternally derived alleles detectable in progeny were used to exclude candidate males, whereas candidate males were not excluded if their visible IF073 alleles were absent in progeny because the candidate male might be heterozygous for a recessive (null) allele. Apparent outcrossed progeny were then assigned fractionally to unexcluded males, weighted by their respective mating opportunities (as defined above) and transmission probabilities at the codominant loci. We chose not to weight candidate males by distance from the maternal parent (cf. Adams et al. 1992), because we lack sufficient empirical data to construct a robust probability distribution for interpair distance.

RESULTS

Genetic Diversity

The allelic diversity of each microsatellite locus is shown in Table 1. At both codominant loci, allele frequencies among ramets and among genets departed significantly from Hardy-Weinberg expectation, but in opposite directions (Table 2). There was a significant excess of homozygotes at IB145, which may be partly attributable to null alleles, based on null frequency estimation by Cervus (Table 1) and observed segregation of null alleles in three maternal individuals (data not shown). In contrast, null alleles appeared to be rare among *fulva*-type hybrids at IF061, and homozygotes were significantly less frequent than expected. All *I. hexagona* plants were null at IF061. (The presence of null alleles reflects the difficulty of isolating microsatellite loci that amplify across the three hybridizing species, and we have noted where this might bias our interpretation.) The isozyme loci also showed no consistent pattern with respect to deviations from Hardy-Weinberg equilibrium. Six of the seven polymorphic isozyme loci assayed by Burke et al. (2000) deviated significantly from Hardy-Weinberg equilibrium; four had excess homozygosity and two had excess heterozygosity. Overall, the average f across loci was 0.037 for the whole population and 0.007 for *fulva*-type ramets. In calculating the average f, we excluded locus PGI-3 because the *I. hexagona* patch and the *fulva*-type hybrids were virtually fixed for alternate alleles, creating a Wahlund effect. These results suggest that there has not been a history of strong inbreeding in this population.

Genotypic linkage disequilibria among *fulva*-type genets, as measured by pairwise correlation coefficients, are shown in Table 3. Approximately half of all pairwise comparisons produced correlation coefficients significantly greater than zero. The correlation coefficients were similar when all *fulva*-

TABLE 2. Deviations from Hardy-Weinberg equilibrium. Inbreeding coefficients calculated by GenePop (Raymond and Rousset 1995) using the method of Weir and Cockerham (1984).

	f				
Locus[1]	All genets	*fulva*-type genets	*fulva*-type ramets	All seedlings	Outcross seedlings
IB145	0.205**	0.215**	0.148**	0.366**	0.288**
IF061	−0.075**	−0.075**	−0.105**	0.216**	−0.023
F16	−0.091	−0.081	−0.070	—	—
FE-1	0.353**	0.389**	0.290**	—	—
6PGD-1	−0.240**	−0.254**	−0.260**	—	—
6PGD-2	0.238*	0.231*	0.164*	—	—
PGI-3	1.000**	1.000**	1.000**	—	—
SKDH	0.220**	0.167**	0.280**	—	—
TPI-1	−0.420**	−0.433**	−0.394**	—	—
Average[2]	0.024	0.020	0.007	0.291	0.133

[1] F16, fructose-1,6-diphosphate; FE, fluorescent esterase; 6PGD, 6-phosphogluconate dehydrogenase; PGI, phosphoglucoisomerase; SKDH, shikimate dehydrogenase; TPI, triose-phosphate isomerase.
[2] Excluding PGI-3; see text.
* $P < 0.05$, ** $P < 0.01$.

TABLE 3. Linkage disequilibrium among *fulva*-type genets as measured by common correlation coefficients. Correlation coefficients were calculated by LinkDos (Raymond and Rousset 1995) and statistical significance was tested using the method of Weir (1979), assuming independence of each comparison.

	TPI-1	SKDH	PGI-3	6PGD-2	6PGD-1	FE-1	F16	IF061
IB145	0.108	0.065	0.086***	0.105*	0.066	0.168***	0.104	0.137***
IF061	0.156***	0.143**	0.101***	0.129*	0.063	0.148***	0.057	
F16	0.103	0.015	0.059	0.150	0.024	0.084		
FE-1	0.192*	0.107	0.225***	0.131*	0.011			
6PGD-1	0.247**	0.021	0.125	0.495***				
6PGD-2	0.224***	0.342***	0.065					
PGI-3	0.095	0.175**						
SKDH	0.130							

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

type ramets were included (data not shown) but more comparisons were statistically significant, presumably because of the larger sample size. Although there was significant linkage disequilibrium between the two codominant microsatellite loci, no disequilibrium was detected within highly selfed fruits, indicating that these loci are not tightly linked physically (data not shown). These results indicate limited recombination among *fulva*-type hybrids as a group, either due to mating patterns or postzygotic selection.

As would be expected, greater resolution of genets was achieved by combining the genetic information of the microsatellite loci with the previous isozyme analysis of Burke et al. (2000). We identified 106 distinct multilocus genotypes, with an average of 1.86 ± 1.83 (SD) ramets per genet and a maximum genet size of 11 ramets. In comparison, Burke et al. (2000) identified 46 genets with an average and maximum size of 4.00 ± 0.50 (SD) and 19 ramets, respectively. The *I. hexagona* patch consisted of four genets constituting a total of 21 ramets; the remaining 102 genets were *fulva*-type hybrids.

Spatial Genetic Structure

The *fulva*-type plants showed pronounced genetic structure at small spatial scales, which declined to zero at approximately five meters. Considering all *fulva*-type ramets, $r = 0.250$ ($P = 0.001$) at two meters and $r = 0.057$ ($P = 0.001$) at four meters. At the microsatellite loci, spatial autocorrelation was greater at IF061 ($r = 0.571$ at two meters, $P = 0.001$) than at IB145 ($r = 0.276$ at two meters, $P = 0.001$). These spatial patterns are in part due to the clonal habit of these plants (Burke et al. 2000). However, after excluding multiple ramets of each genet, the strength of autocorrelation was somewhat reduced but remained highly significant. For all microsatellite and isozyme loci combined, $r = 0.188$ ($P = 0.001$) at two meters and $r = 0.048$ ($P = 0.001$) at four meters (Fig. 1). For IF061, $r = 0.476$ ($P = 0.001$) at two meters and $r = 0.114$ ($P = 0.001$) at four meters; for IB145, $r = 0.258$ ($P = 0.001$) and $r = 0.080$ at four meters ($P = 0.001$).

The mean radius of clonal patches was less than 1.2 m in

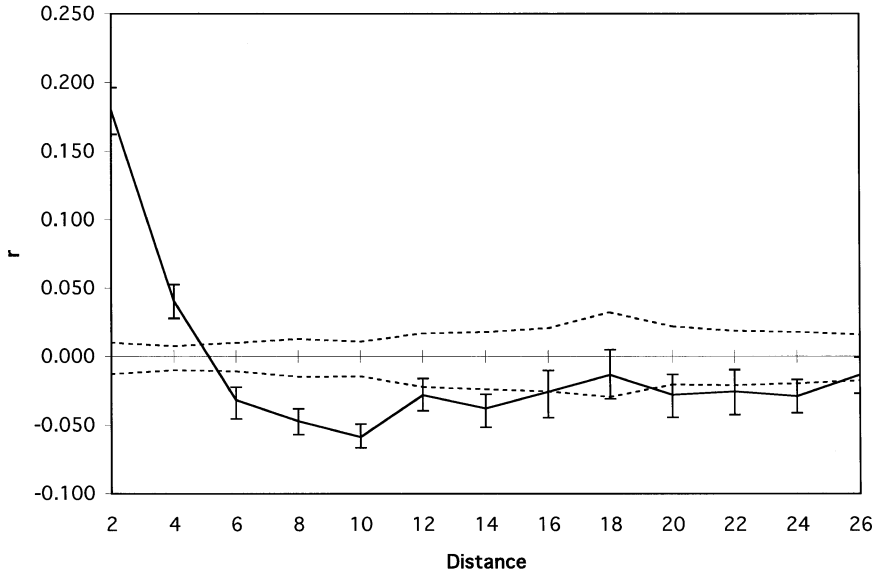

FIG. 1. Spatial autocorrelation across all microsatellite and isozyme loci among *fulva*-type genets, as calculated by GenAlEx (Peakall and Smouse 2001). In the computation of this statistic, each genet is represented only once and its spatial coordinates are defined by the average x- and y-coordinates of all ramets of the genet. The solid, bold line represents the autocorrelation coefficient and the dashed lines represent the bootstrapped 95% confidence interval.

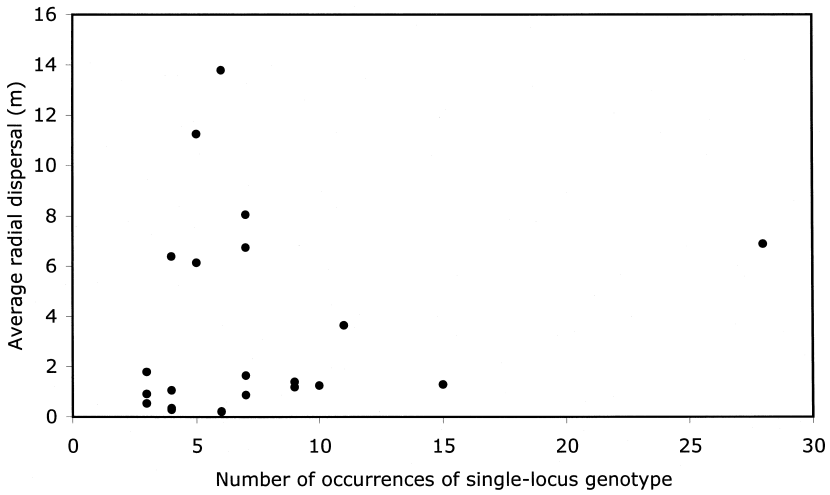

FIG. 2. Average radial dispersal of all single-locus genotypes of the microsatellite loci IB145 and IF061 that are present in three or more individuals. Average radial dispersal is the distance in meters from the center of each genet to the center of all genets sharing the same single-locus genotype.

all but one case, in which four ramets with the same multilocus genotype were found widely dispersed in the population (mean radial distance of 20.3 m). This extreme outlier was excluded from the analysis. The average of the mean radial distances within clones was 0.56 meters and between clones was 11.79 m, a highly significant difference by two-tailed *t*-test ($P < 0.0001$). When the effect of clonality was removed by including only one ramet per genet at its center point, single-locus genotypes and alleles were still frequently localized to very small areas. Of the 23 single-locus microsatellite genotypes present in three or more genets, 15 (65.2%) had a mean radial dispersal of <2 meters (Fig. 2). Of the 16 alleles present in three or more genets, six (37.5%) had a mean radial dispersal of <3 m.

Table 4 shows the probability of the *fulva*-type genotype array for different values of *K*, the number of admixed groups, as calculated by Structure (Pritchard et al. 2000). The value of α, which reflects the proportion of individuals of mixed ancestry (Pritchard et al. 2000), and the posterior probability of *K* are also shown for each run (Table 4). The parameter α was consistently low (0.038–0.055), indicating that there is little intermixing between groups (Pritchard et al. 2000). The posterior probability of *K* was highest for $K = 5$. These results showed no appreciable change across multiple independent runs of the simulation. For each value of *K*, ramets were assigned to the group that formed the largest part of their inferred ancestry (see Materials and Methods) and their spatial locations marked accordingly. Figure 3 illustrates the location of ramets in each group for $K = 5$. Individual ramets assigned to a group based on a fraction of ancestry less than 0.8 are marked with an X in Figure 3. For the studied values of *K*, these clusters were strongly localized. For example, the spatial autocorrelation of group identity for $K = 5$ was 0.766 at 2 m and 0.380 at 4 m.

TABLE 4. Structure (Pritchard et al. 2000) simulation results for *fulva*-type genets for given values of *K*. The parameter α reflects the proportion of individuals of mixed ancestry. Parameter estimates are obtained by Markov-chain Monte Carlo simulation with 100,000 runs after a 10,000 run burn-in. The posterior probability of *K* is calculated using Bayes's rule with a uniform prior probability for all *K*. See text for details.

Number of groups (*K*)	ln(probability of *K*)	α	Posterior probability of *K*
2	−1376.0	0.055	0.000
3	−1324.4	0.047	0.000
4	−1289.0	0.041	0.000
5	−1264.0	0.041	0.953
6	−1267.0	0.040	0.047
7	−1299.6	0.040	0.000
8	−1383.3	0.039	0.000
9	−1429.8	0.038	0.000
10	−1614.0	0.039	0.000

Genetic Correlations with Phenology

Overlapping phenology was not associated with increased genetic relatedness of potential mating pairs. Flowering ramets were not more related to each other on average than to the population as a whole ($R = 0.1016 \pm 0.0882$ SE, $P > 0.1$) based on the combined microsatellite and isozyme data. Moreover, among flowering individuals, the order of flowering did not produce genetic correlations between potential mating pairs. The Mantel regression of pairwise difference in date of first flower on genetic distance was not significantly different from zero ($r = 0.10$, $P = 0.140$). The Mantel regression of total pairwise mating opportunities on genetic distance was also not significant ($r = -0.22$, $P = 1.000$). These regressions were also not significant when considering *fulva*-type hybrids alone (data not shown). The probability per flower that pollen drawn at random from coflowering pollen donors would produce an apparent outcrossed zygote was generally high (mean = 75%).

Mating System

A total of 325 of 494 seeds germinated and were successfully genotyped for at least one microsatellite locus (65.8%). The population mean apparent selfing rate was high ($s = 0.677$), but the distribution of selfing estimates was

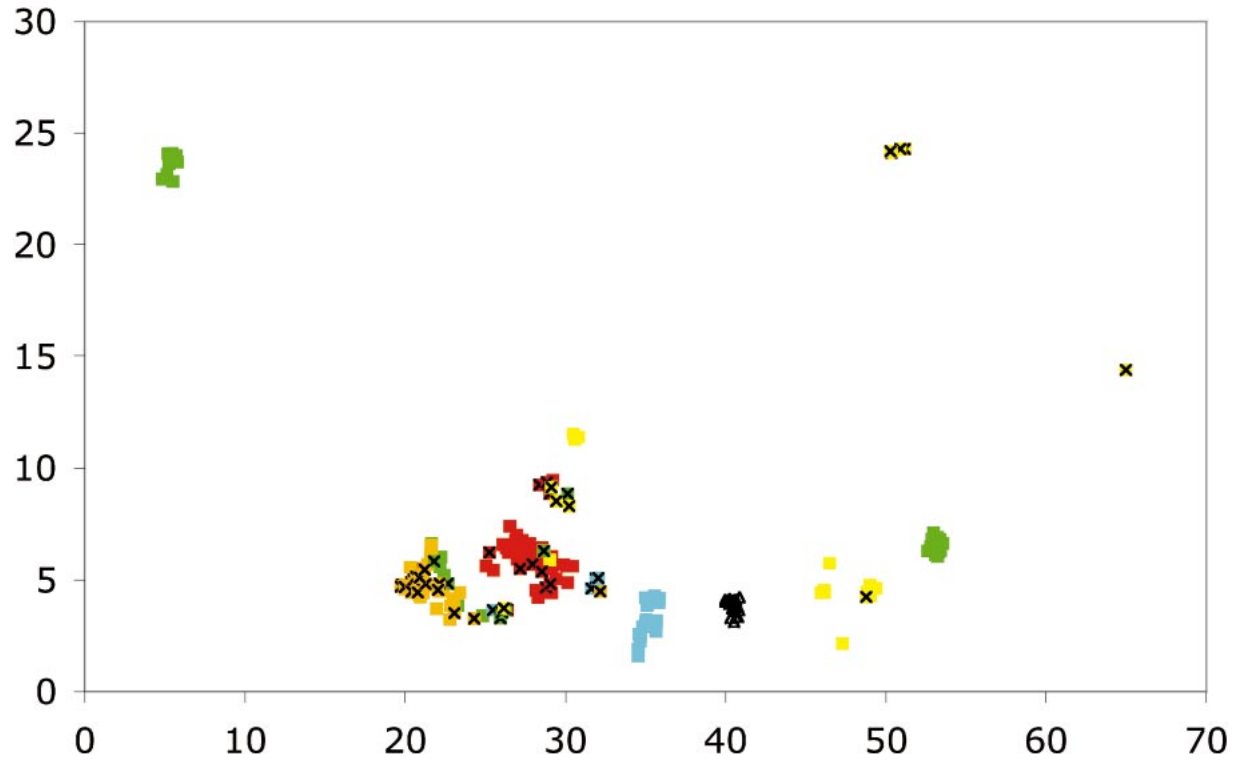

FIG. 3. Map of ramets by group identity as determined by Structure (Pritchard et al. 2000) for $K = 5$. Each color represents a specific group. Solid, colored squares represent individuals with an estimated percent ancestry of ≥80% from the specified group. Squares with an X derive the greatest fraction of their ancestry from the specified group but the estimated percentage is <80%; these individuals are considered to be of mixed ancestry. *Iris hexagona* ramets are represented by open triangles. Axes are in meters.

bimodal, with most fruits highly selfed or highly outcrossed. All but three seeds produced by *I. hexagona* fruits were compatible with selfing, but because all flowering *I. hexagona* had the same microsatellite genotypes, outcrossing among these genets could not have been detected. We can conclude only that mating between the two phenotypic classes is rare. Considering only the *fulva*-type plants, the mean apparent selfing rate was considerably lower ($s = 0.478$).

Not surprisingly, given the rate of selfing in this population, the total progeny array was significantly inbred (Table 2). Even among outcross progeny, there was a significant excess of homozygotes at IB145 ($f = 0.288$, $P < 0.0001$) but not IF061 ($f = -0.023$, $P = 0.214$). However, three fruits were heterozygous for a null allele at IB145 as evidenced by their segregation patterns, which inflates the IB145 inbreeding coefficient considerably. Excluding these fruits reduces f to 0.146 but the estimate remains significantly greater than zero ($P < 0.0001$).

Significant linkage disequilibrium between these loci ($P = 0.004$) was maintained in the outcross progeny array, which is probably due in part to the low number of fathers per fruit. By genetic exclusion, the minimum number of fathers was one to three per fruit, with a mean of 1.4.

Patterns of Paternity

The number of outcross progeny for which a single, unique candidate male was identified was 10 of 105 (9.5%) detectable outcross events. All censused males were excluded for 57 (54.3%) of these detectable outcross events; these progeny were attributed to pollen flow from outside the population. The remaining 38 outcross events (36.2%) were assigned fractionally to all unexcluded candidates as described in Materials and Methods.

The average number of seeds sired per flowering individual was 1.88 ± 1.76 (SE). Eighteen of 24 flowering individuals were assigned outcross paternity (75%); this value is likely to be substantially inflated by the fractional method of paternity assignment (Devlin et al. 1988). The distance between mating pairs was relatively high, and a large fraction of outcross pollen was derived from outside the censused population (i.e., ''gene flow'' distance class, Fig. 4). Although fractional paternity assignment might in principle distort the true distance of pollen movement, for example by overestimating the contribution of more distant candidate males, this bias is unlikely to strongly affect these results. This is due to the strong spatial structure of the population, a consequence of which is that unexcluded candidate males were generally in proximity, in which case the weighted average of the group of pairwise distances was roughly the same as each individual distance. However, this spatial structure is itself a source of bias, because matings over shorter distances are less likely to be identified as outcrossing. Nonetheless, the linkage disequilibrium among marker loci and the strong correlation of selfing within fruits (cf. Cruzan et al. 1994) argue that the available markers provide a suitably accurate estimate of the mating system. Regardless of this uncertainty

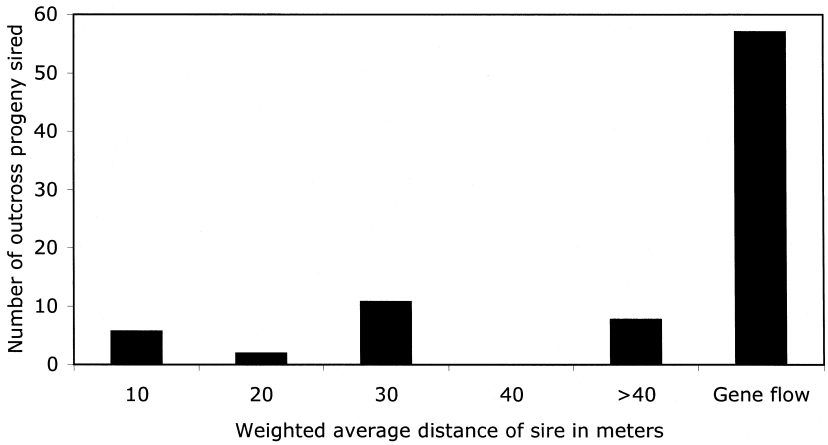

FIG. 4. Frequency of detected outcross pollen movement by distance class. Pollen movement is based on fractional paternity assignment as described in Materials and Methods. Therefore, assignment of individual outcross events is made fractionally to each distance class according to the weights of each candidate father in that distance class.

with respect to the frequency distribution of pollen movement, it is clear that a significant portion of pollen is transported over scales of tens of meters.

DISCUSSION

Comparison of Adult and Progeny Structure

In this study, we described patterns of genetic structure in a Louisiana iris hybrid zone at adult and seed life-history stages. Overall, there was greater genotypic diversity among *fulva*-type hybrids at Young's Coulee than we anticipated based on previous work (Burke et al. 2000). The relatively large number of genotypes (102 multilocus genotypes among 176 mapped ramets) and small size of clones (1.73 ± 1.56 SD ramets per genet) among *fulva*-type plants indicate that recruitment from seed is frequent. Genetic structure was nonetheless very strong at short spatial distances (<5 m). This structure was partially independent of clonality, as evidenced by (1) the significant spatial autocorrelation among genets, and (2) the localization of many single-locus genotypes and alleles. These observations are not expected based on clonal propagation per se, as there is no reason that genets separated by considerable distances should not share alleles if the original seedlings were derived from outcross pollen flow. Instead, the data indicate low effective dispersal of both pollen and seed, probably in conjunction with selfing.

Although the spatial distribution of alleles of adults indicates a small genetic neighborhood, genotypic proportions show no evidence of historically strong inbreeding: the mean f across loci (excluding PGI-3) among *fulva*-type hybrids is 0.007. Using Ritland's equilibrium-model estimation of inbreeding depression (Ritland 1990), we obtain an estimate of inbreeding depression between seedling and adult life-history stages that approaches unity ($D = 0.987$). Although it is possible that the mating system observed in this reproductive season is atypical, studies of pure *I. fulva* have also shown a mixed-mating system with bimodal selfing rates (Cruzan et al. 1994). The population outcrossing rate estimated by Cruzan et al. (1994) was 0.47–0.67, depending on estimation method, values close to our own ($t = 0.52$). Thus, it appears that in this population, *fulva*-type hybrids have a mating system similar to that of the pure species. A second concern is whether an assumption of equilibrium between mating system, selection, and adult genetic structure is appropriate for a given population, especially for potentially long-lived organisms. Although theory indicates a fairly rapid approach to equilibrium under the mixed-mating model (Clegg 1980), our results indicate that the assumption of random mating among the outcross fraction is violated due to significant deviations from Hardy-Weinberg and linkage equilibrium among outcross progeny. We therefore treat the quantitative estimate of D with appropriate skepticism, but the qualitative result is not altered by this uncertainty. That is, homozygote progeny are not present in the adult population in proportion to the rate at which they are formed, by a considerable margin. Whether this is due to traditional mechanisms of inbreeding depression (e.g., exposure of deleterious recessive alleles or loss of heterotic interactions) or due to genetic factors peculiar to hybrid zones (such as Dobzhansky-Muller epistatic interactions) is unknown.

Not surprisingly, linkage disequilibrium was moderately strong among ramets in the population as a whole. We expected to find some level of linkage disequilibrium because it is an immediate consequence of the mixing of genetically differentiated groups, particularly in interspecific hybrid zones where the action of recombination may be opposed by natural selection against particular hybrid genotypes (Dobzhansky 1970). Linkage equilibrium is also opposed by selfing and biparental inbreeding (Haldane 1949). However, it is somewhat surprising that even among the *fulva*-type hybrids there is a low rate of recombination.

When we used Structure to identify groups of *fulva*-type ramets in approximate Hardy-Weinberg and linkage equilibrium, the inferred groups were strikingly localized in space. For example, the red and orange groups represent large groups of genets between which there is a narrow border a few meters wide. This boundary appears at $K = 2$ and remains largely intact across a wide range of K (data not shown). Whether this is due to demographic factors such as limited seed dispersal or postzygotic selection is unknown, but the

proximity of the groups and the fact that plants in each group flowered simultaneously (data not shown) suggest a role for the latter. The blue group is also noticeably confined, but it appears to have greater clonal structure than on average. For $K = 5$, the cluster consists of eight genets and 24 ramets, including two clones of five and 11 ramets. Interestingly, no plants in this group flowered during the study year. Other groups identified by Structure show less pronounced spatial localization. We draw two general conclusions from these results: (1) recombination among *fulva*-type hybrids has occurred within roughly contiguous blocks over restricted spatial scales, and (2) recombination occurs infrequently between these groups. Although these results were presaged by and are consistent with the other spatial analyses we performed, the Structure analysis integrates a greater amount of information and identifies specific genets that make up genotypic clusters or are of mixed ancestry.

Inference of the true value of *K*, the number of groups, is based primarily on two factors, the posterior probability of the dataset given various hypothesized values of *K*, and the biological context in which the groups are interpreted (for a discussion see Pritchard et al. 2000). Ideally, the calculated probability of the data given *K* has a clear maximum for some biologically plausible value of *K*, which should be a small number for most scenarios of admixture and hybridization. In the present case, the posterior probability was highest for $K = 5$. However, violations of the clustering model are possible (e.g., inbreeding and null alleles) and these will tend to inflate the estimate of *K*. It is probably more useful to consider a range of plausible *K* and the commonalities within that range with respect to the spatial patterns of recombination and admixture. It should be noted that when there is little real structure in a sample, the program tends to identify groups approximately equal in size and the value of α is unstable within runs (Pritchard et al. 2000). In our analysis, neither of these phenomena was observed (data not shown), which provides additional support for the detected structure.

One caveat of this analysis is the limited resolution afforded by the available genetic markers. Although our results are based on a moderate number of loci, effectively eight, given that the *fulva*-type genets are virtually fixed at PGI-3, this number of independent markers argues forcefully against free recombination at least in a large portion of the genome. A separate issue is the effect of low polymorphism at some loci on the power of our study to detect existing structure. Although a more distinct spatial pattern of genetic structure may well be detected with the addition of more polymorphic markers, the reverse finding—that there is less spatial structure—seems highly improbable. Low polymorphism is inherently conservative with respect to the statistical analysis of genetic structure because it makes genetically distinct individuals appear similar.

Effect of Phenology on Genetically Correlated Mating

Patterns of adult genetic structure in hybrid zones reflect both the genetic composition of the population founders and subsequent patterns of effective mating. Whether mating patterns are due to prezygotic or postzygotic factors requires direct investigation of the processes that determine mating pairs. In this study we examined the phenology of the flowering cohort to determine how variation in the pollen pool might structure mating pairs. Because the phenology of *I. fulva* × *I. brevicaulis* hybrids is heritable under greenhouse conditions (A. Bouck, unpubl. data), we hypothesized that coflowering individuals in hybrid zones may be more related to each other on average than to the population as a whole. If so, this would result in genetic correlations among mating pairs, thereby producing clusters of progeny with similar genotypes if seed dispersal is limited. Although differences in microhabitat variables such as soil moisture and shade are also expected to contribute to phenology, previous studies (Cruzan and Arnold 1993; Johnston et al. 2001) found significant associations between genotype and microhabitat in another Louisiana iris hybrid zone. If generalizable, this covariation should tend to reinforce the genetic correlation of mating pairs due to phenology rather than dilute it. However, in this study we did not find any evidence that coflowering individuals were genetically correlated at the population level. Yet, average values of genetic relatedness or distance may be misleading—although on average coflowering individuals were not genetically correlated, coflowering near neighbors typically shared similar or identical microsatellite genotypes. Thus, if as suggested by studies of pollinator behavior (Wesselingh and Arnold 2000) matings are frequently between near neighbors, biparental inbreeding would be accordingly more common. It is also possible that other potential mechanisms of assortative mating not investigated in this study, such as quantitative variation in floral morphology among *fulva*-type hybrids, may be important in Louisiana iris hybrid zones.

Although phenology did have an effect on the genotypic distribution of progeny in an *I. fulva* × *I. brevicaulis* hybrid zone (Cruzan and Arnold 1994), the mean flowering time of these species differs by several weeks and—as has been observed in controlled crosses—there is segregating variation for relative flowering time among their hybrids (A. Bouck, unpubl. data). Since the species *I. fulva* and *I. hexagona* have similar phenologies in nature (Arnold et al. 1993), it is possible that genetic variation for flowering time could only come from the *I. brevicaulis* genome. Thus, there may be insufficient segregating variation for phenology in this population to effect a correlation between genetic similarity and flowering time.

Gene Flow between Iris hexagona and fulva-type Hybrids

The low level of pollen flow between *I. hexagona* and *fulva*-type hybrids (about 1%) accords with our expectation based on previous work (Hodges et al. 1996). Furthermore, when the *I. hexagona* genets are included in the Structure analysis, only one *fulva*-type genet with a clearly recombinant genotype clusters with them, and only for some values of *K* (data not shown). This indicates that intermediate genotypes are rare among the adult population as well. Collectively, these observations justify the analysis of *fulva*-type hybrids as a distinct subpopulation. The rarity of phenotypic and genotypic intermediates is characteristic of Louisiana iris hybrid zones, a pattern that appears to be stable based on multiyear and multisite data (Cruzan and Arnold 1993, 1994). This

probably reflects both the extensive barriers to F_1 seed formation in these species and some form of hybrid breakdown. Although experimental studies have documented high vegetative and sexual fitness of F_1s (Burke et al. 1998a, Emms and Arnold 1997), viability selection against intermediate genotypes in the F_2 generation has also been documented (Burke et al. 1998b).

The Distribution of Progeny Genotypes

Although the observed rate of outcrossing was insufficient to break down genotypic disequilibria at the microsatellite loci within the overall progeny array, paternity assignment indicated that pollen did move over substantial distances. Approximately 70% of assigned outcross paternity was to individuals >20 m from the maternal plant. Furthermore, a large fraction of these outcross progeny (54.3%) were fathered by individuals outside the censused population, as determined by genetic exclusion. The relatively high dispersal distance of pollen could be interpreted in two ways. One possibility is that because the probability of detecting outcross events between near neighbors is generally lower, the true frequency of pollen flow over short distances is higher than evidenced by these data, and thus actual outcrossing rates are underestimated. This view is supported by observations of pollinator behavior in this system (Emms and Arnold 2000; Wesselingh and Arnold 2000), which show a strong bias toward near-neighbor transitions. However, the alternative explanation, that long-distance pollen flow is fairly common, is supported by the emerging generality of this finding in plant paternity analyses (Ennos 2001) and by evidence of introgression via pollen flow between disjunct populations of Louisiana iris (Arnold et al. 1992).

The observed patterns of pollen movement and negligible inbreeding coefficients among adults are contrary to the strong spatial structure of genets, in which single-locus genotypes and individual alleles are frequently limited to small regions of the population. Our results also imply a low rate of seed dispersal, which is somewhat surprising given that *I. fulva* and *I. hexagona* seeds have a corky, buoyant seed coat that is presumably adapted for water dispersal. It is possible that the patchiness of adult genotypes reflects spatially episodic recruitment of seedlings dominated by a few nearby maternal individuals. Alternatively, there may be undetected environmental variables that favor particular genotypes in different patches. These hypotheses are purely conjectural; the simplest explanation remains that most seeds are dispersed near the maternal individual despite the potential for long-distance transport by water.

A difficulty with our analyses that merits attention is the presence of null alleles at the microsatellite loci, which obscure the true genotype of individuals and may lead to false inferences regarding genetic structure and paternity. In our sample there was a high frequency of null alleles at IB145 among *fulva*-type individuals, and all *I. hexagona* individuals were null at IF061. However, the estimated null frequency at IB145 was much lower within the cohort of flowering individuals. Furthermore, *I. hexagona* are a priori not expected to mate frequently with *fulva*-type plants (e.g., Hodges et al. 1996) and are also quite distinct at IB145 and IF073 in this population (data not shown). Thus, we do not believe that null alleles significantly impact the paternity results. With respect to the spatial analyses, null alleles at IB145 can cause both positive and negative deviations from actual genetic distance, as defined by Smouse and Peakall (1999), for individual pairwise comparisons. It is therefore difficult to assess the cumulative effect of nulls on the spatial autocorrelation statistic at this locus, but the impact on spatial autocorrelation across all loci should be minimal. However, null alleles should tend to inflate the estimation of K in the Structure analysis by creating false deviations from Hardy-Weinberg equilibrium, and we caution that this bias may be present in our data.

Despite these caveats, the overall patterns of paternity and genetic structure among *fulva*-type hybrids indicate that neither outcrossed progeny nor homozygous selfed progeny are recruited at the frequency with which they are formed. Rather the contrast between adult and progeny structure suggests that progeny with genotypes similar to adults are the most successful. It bears pointing out that this conclusion is qualitatively unchanged if actual outcrossing rates differ somewhat from our estimates, either due to inherent ambiguities in estimation or to natural variation from year to year. Although we do not know the basis of postzygotic selection in this population, such mechanisms have been an area of active investigation in Louisiana irises. Marker-based studies of experimental hybrids have uncovered selection against certain hybrid genotypes in the F_2 (Burke et al. 1998b) and BC_1 generations (A. Bouck, unpubl. data), as indicated by segregation distortion of marker loci and negative epistatic interactions between loci. In addition to nuclear genetic incompatibilities, cytonuclear incompatibilities have been identified in these hybrid classes (Burke et al. 1998b), which might contribute to the maintenance of linkage disequilibrium and heterozygosity in this hybrid zone.

These conclusions have important consequences for the establishment of recombinant lineages that are more fit than the parental types in some habitats. Grant (1971) and others have pointed out the Sisyphean dilemma of hybrid novelty, which is that adaptive gene combinations brought into existence by recombination will be undone by the same process. Our results suggest that partial reproductive isolation can occur over small spatial scales relative to pollen flow in Louisiana iris hybrids. Thus, in this system, life-history characteristics such as selfing and asexual propagation do appear to contribute to the stabilization of hybrid lineages as predicted by theory (e.g., Grant 1971; McCarthy et al. 1995), but postzygotic selection against subsequent recombination is also significant. Future research will need to address a separate but equally important question, which is whether novel recombinant types can spread geographically and demographically from their points of origin.

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